NEW MOLECULAR COMPLEXES PRESENTING HIGH AFFINITY BINDING WITH RESPECT TO MONOCYTE DERIVED CELLS AND THEIR USES IN THERAPY

The invention relates to new molecular complexes presenting high affinity binding with respect to monocyte derived cells and their uses in therapy.

Blood monocytes in physiological conditions leave the blood stream flow to reach tissues where they differentiate into resident macrophages (for example: lung macrophages, kupffer cells in liver, skin macrophages, osteoclasts in bone, microglial cells in brain ...), or into professional antigen presenting cells (for example : dendritic cells in peripheral tissues or lymphnodes, Langerhans cells in skin ...).

Differentiation of blood monocytes can also be achieved *ex vivo* under defined culture conditions (see applications WO94/26875, WO96/22781, WO97/44441, WO99/13054); however, the macrophages or the dendritic cells obtained in culture do not achieve tissue specificity similar to the one obtained *in vivo*).

Furthermore, the induction of an immune response has been documented when the antigens are known. However, regarding the induction of an immune response towards unknown antigens (particularly tumor antigens), a targeting of these antigens to specific receptors of the antigen presenting cells is required ; this is an objective of the present invention.

One of the aims of the invention is to provide monocyte derived cells which have acquired a tissue specificity.

Another aim of the invention is to provide an *ex vivo* method for stimulating cellular and/or humoral immune responses against unknown components of a tumor tissue extract.

Another aim of the invention is to provide *in vivo* specific cellular and/or humoral immune responses against unknown component of tumor tissue extract.

All these aims are achieved through the invention, which gives access to new molecular complexes having high affinity with tissue extracts on the one hand, and high affinity with monocyte derived cells on the other hand.

More precisely, the invention relates to a molecular complex between a tissue extract containing at least one known component and unknown components and a molecular vector comprising a particle bearing polypeptides and/or sugars, said molecular vector being able to recognize :

- said known component of said tissue extract, and
- a phagocytic receptor of monocyte derived cells,

with the proviso that polypeptides are different from antibodies.

The expression "*known component*" means identified tissue antigens, polypeptides or oligosaccharides or an hapten expressed or transfected on the cell membrane of tissues or tumors.

The expression "*unknown component*" means "complex mixture of proteins and saccharides present in cellular extracts of tumors or tissues (lysates, apoptotic extracts,...)

The expression "*molecular vector*" corresponds to a carrier of molecular structure.

The expression "*recognize said known component of said tissue extract*" means that it presents a high affinity and/or avidity ($>10^{-6}$ M) for said component.

The expression "*recognize a phagocytic receptor of monocyte derived cells*" means that it is a ligand for such receptor.

The expression "*polypeptides are different from antibodies*" means that they are not monoclonal or polyclonal antibodies with Fc and Fab parts.

A phagocytic receptor of monocyte derived cells is a receptor such that, when interacting with a ligand, in this case, the molecular complex, it initiates uptake of said ligand.

The phagocytic status means that the monocyte derived cells have gained, after a few days of culture, for instance, about 4 to about 10 days, a high

phagocytic activity. (This phagocytic activity can be visualized and quantified by measuring, for instance under the microscope, the uptake of yeast particles).

The expression "*monocyte derived cells (or MDCs)*" designates macrophages or dendritic cells derived from blood monocytes.

According to an advantageous embodiment, the invention relates to a molecular complex wherein the molecular vector comprises a particle bearing polypeptides and/or sugars such that :

- at least one of the said polypeptides and/or sugars recognizes said known surface component of the tissue extract,
- at least one of the said sugars and/or polypeptides recognizes phagocytic receptors of monocyte derived cells such as receptors for mannose or for oligosaccharides or Fc receptors of monocyte derived cells.

There are thus four different possibilities :

1) at least one of the said polypeptides of the particle can recognize a known component of the tissue extract and at least one of the said polypeptides of the particle can recognize a phagocytic receptor of monocyte derived cells,

2) at least one of the said polypeptides of the particle can recognize a known component of the tissue extract and at least one of the said sugars of the particle can recognize a phagocytic receptor of monocyte derived cells,

3) at least one of the said sugars can recognize a known component of the tissue extract and at least one of the said sugars of the particle can recognize a phagocytic receptor of monocyte derived cells,

4) at least one of the said sugars can recognize a known component of the tissue extract and at least one of the said polypeptides of the particle can recognize a phagocytic receptor of monocyte derived cells.

The nature of the bond between the sugar and the known component is formed of hydrogen, Vanderwaals, hydrophobic interactions and salt bridges.

The nature of the bond between the sugar and the monocyte derived cells is mainly hydrogen, Vanderwaals, hydrophobic interactions and salt bridges.

The nature of the bond between the polypeptides and the known component is mainly hydrogen, Vanderwaals, hydrophobic interactions and salt bridges.

The nature of the bond between the polypeptide and monocyte derived cells is mainly hydrogen, Vanderwaals, hydrophobic interactions and salt bridges.

In an advantageous embodiment of the molecular complex of the invention, the molecular vector comprises or is a particle of about 0,1 to about 2 μm of biocompatible polymer comprising :

- surface polypeptides and/or sugars, preferably covalently linked to the surface of said particle, with said surface polypeptides and/or sugars recognizing said known component of the tissue extract, and
- mannosylated residues recognizing the mannose or oligosaccharide receptors of monocyte derived cells.

According to an advantageous embodiment, in the molecular complex of the invention, the tissue extract comprises macroscopic fragments or killed or irradiated or haptenised human or animal tumor cells such as lysates or apoptotic bodies, or killed pathogens, such as viruses or bacteria.

According to an advantageous embodiment, in the molecular complex of the invention, the polypeptide of the particle recognises one known epitope of the tissue extract chosen among known tumor antigens such as (tumor peptide antigen) MelanA/MART-1, MAGE, BAGE, GAGE families, MUC, EGF-R, ERB-2, PSA, PSMA, HSP70, CEA, P53, RAS, Tyrosinase, gp100,....

According to another advantageous embodiment, in the molecular complex of the invention, the tissue extract comprises normal tissue parts such as tissue membranes, tissue factors, tissue proteins, macroscopic fragments of tissue such as lysates or apoptopic bodies, said tissue being originating from any part of human or animal body or cellular extracts thereof, in particular from thymus, lung, pancreas, cartilage, endothelium, neuromuscular junctions,

prostate, sexual organs, bladder, muscles, peripheral nerves, CNS extracts, spleen, liver, bone, heart, skin cells.

In the molecular complex of the invention, the polypeptide and/or sugars of said particle form(s) high affinity binding with any component of said tissue extract.

In the molecular complex of the invention, the polypeptide and/or sugars of the particle form(s) high affinity binding with a phagocytic receptor of a monocyte derived cell.

The expression "*high affinity binding*" means that the affinity constant Ka is equal to or higher than 10^6 M or the equilibrium dissociation constant K_D is equal to or lower than 10^{-6} M.

According to an advantageous embodiment, the monocyte derived cells recognized by the molecular complex of the invention are macrophages, dendritic cells, or antigen presenting cells.

The invention also relates to monocyte derived cells such as prepared according to a process comprising the step of contacting monocyte derived cells with a molecular complex according to the invention.

The invention also relates to monocyte derived cells such as prepared according to a process comprising contacting monocyte derived cells with a molecular complex according to the invention, under conditions enabling phagocytosis of said molecular complex by said monocyte derived cells, intracellular degradation and processing of the known and unknown components of the tumor tissue extract and the presentation of said known and unknown components on the peripheral membrane of the monocyte derived cells together with MHC 1 and MHC II molecules.

The monocyte derived cells are immature dendritic cells for the phagocytosis, which then mature for the induction of immune response.

The invention also relates to monocyte derived cells such as prepared according to a process comprising contacting monocyte derived cells with a

molecular complex as described above, under conditions enabling phagocytosis of such molecular complex by the monocyte derived cells.

The monocyte derived cells are non-activated macrophages (4/8 days of culture).

The invention also relates to an *ex vivo* method for stimulating cellular and/or humoral immune responses against unknown components of a tumor tissue extract comprising contacting monocyte derived cells with a molecular complex according to the invention, under conditions enabling phagocytosis of said molecular complex by monocyte derived cells, intracellular degradation and processing of the known and of unknown components of the tumor tissue extract and the presentation of said known and unknown components on the peripheral membrane of the monocyte derived cells, together with MHC I and II molecules.

The invention also relates to a method of inducing *in vivo* specific cellular and/or humoral immune responses against unknown components of tumor tissue extract comprising injections of a molecular complex according to the invention, for instance by intramuscular, subcutaneous, local or intravenous route.

According to an advantageous embodiment, said method of inducing *in vivo* specific cellular and/or humoral responses against unknown components of a tumor tissue extract, comprises sequential and/or simultaneous injections of monocyte derived cells presenting known and unknown components of said tumor tissue extract, together with MHC I and II molecules, as defined above, and of molecular complexes as described above.

The invention also relates to a method for conditioning *ex vivo* monocytes derived cells, and preferentially macrophages, for them to acquire tissue specificity, comprising contacting monocyte derived cells with a molecular complex according to the invention, under conditions enabling phagocytosis of said molecular complex by the monocyte derived cells.

The expression "*conditioning ex vivo human monocyte derived cells*" means that after phagocytosis of specific tissue extracts, the MDCs acquire characteristics of the corresponding tissue macrophages.

The expression "*acquire tissue specificity*" means that when the MDCs are injected *in vivo*, they will (concentrate) accumulate preferentially in the corresponding tissue.

The invention also relates to a method of treatment of diseases involving accumulation of conditioned monocyte derived cells as described above in specific tissue to induce tissue repair and/or regeneration comprising :

- either simultaneous and/or sequential injections of monocyte derived cells and of a molecular complex according to the invention, under conditions enabling phagocytosis,

- or injection of the monocyte derived cells which have previously phagocytosed a molecular complex according to the invention.

The expression "*accumulation of conditioned monocyte derived cells*" in a tissue means that, after systemic injection, at least 10% of the cells injected accumulate in the tissue within 24 h.

In the invention, the monocyte derived cells which are advantageously involved are human monocyte derived cells.

By way of example, the diseases which can be treated by the method of the invention are tissue/organ destruction or degenerative diseases, when tissue repair is required (skin, bone, nerve, neuromuscular regeneration).

The invention also relates to pharmaceutical compositions comprising, as active substance, monocyte derived cells which have been contacted with a molecular complex according to the invention, under conditions enabling phagocytosis of said molecular complex by monocyte derived cells, intracellular degradation and processing of the known and of unknown components of the tumor tissue extract and the presentation of said known and unknown components on the peripheral membrane of the monocyte derived cells, together with MHC I and II molecules.

The invention also relates to pharmaceutical compositions comprising, as active substance, monocyte derived cells, and preferentially macrophages, which have been contacted with a molecular complex according to the invention, under conditions enabling phargocytosis of said molecular complex by the monocyte derived cells.

EXAMPLE 1 : Application to a human melanoma tumor:

Apoptotic bodies are generated from a human melanoma cell line M17 by UV irradiation. They are added in basic medium to microparticles of 0.2 to 2 μm with covalently linked annexin V polypeptides and mannosyl residues. Annexin V presents a high affinity for phosphatidyl serine residues expressed on apoptodic bodies.

The microparticles contain a magnetic core and the molecular complexes (tumor apoptotic bodies - microparticles) are isolated on magnets. A working bank of molecular melanoma complexes is constituted and kept frozen.

Patients with metastatic melanoma are injected into 4 subcutaneous sites, one intradermal site plus one intravenous site with the defrost preparation.

The injections are repeated after 2 weeks and again one month later. Interaction with dendritic cells is occurring locally in the patient.

The induction of a specific immune response against the melanoma tumor is documented by humoral and cellular T responses against the known MAGE and MelanA/MART antigens expressed by the M17 cell line. The global antitumoral effect is shown by shrinkage ($> 50\%$) of subcutaneous metastases, this response requires immune activation against multiple melanoma tumor antigens or than the targeted antigen.

EXAMPLE 2 : Application to tissue repair in a murine model:

Microparticles of 0.2 to 2 μm size presenting at their surface mannosyl residues are added to a suspension of killed murine hepatocytes, and molecular complexes are formed.

Macrophages are obtained by differentiation of murine bone marrow cells in culture and labelled with indium or an emittor of positons (example: Fluor 18).

These macrophages are grown for 16 h in the presence (a) or the absence (b) of molecular complexes.

Two millions of these macrophages are injected intravenously to the mice. After 2 hours, the biodistribution of the macrophages in the animal tissues is measured by gamma counting or PET-scan (SMV International). In case (a), 90% of the macrophages injected are in the liver while in case (b), only 20% of the macrophages are in liver. This indicates that the macrophages grown in the presence of the molecular complexes have gained a liver tissue specificity. If necrosis of the liver is previously induced, a fast regeneration is seen a few days after macrophage injection.